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Goldenberg

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[54] TUMOR LOCALIZATION AND THERAPY WITH LABELED ANTI-CEA ANTIBODY

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[52] U.S. Cl. 424/1; 128/1.1; 128/659; 424/9

[58] Field of Search 424/1, 9; 128/659, 1.1

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[57] ABSTRACT

Improved methods are provided for using radiolabeled antibodies to carcinoembronic antigen (CEA) to locate, diagnose and stage CEA-containing tumors by external photoscanning, whereby significantly increased resolution, convenience and/or efficiency of operation may be achieved. A method is provided for using highly specific radiolabeled anti-CEA antibodies for tumor therapy. An injectable composition and radiolabeled antibodies are provided for use in the method of the invention.

39 Claims, No Drawings

TUMOR LOCALIZATION AND THERAPY WITH LABELED ANTI-CEA ANTIBODY

BACKGROUND OF THE INVENTION

It is known that radiolabeled antibodies to carcinoembronic antigen (CEA) can be used to localize tumors. U.S. Pat. No. 3,927,193, to Hansen et al, discloses such a method, but provides examples of its use only in animals. The method described in this patent does not explain how tumors may be visualized in a situation where radioactivity is also present in other sites of the body, such as blood, other body fluids and certain tissues, particularly heart and liver, which can prevent precise discrimination of the radioactivity associated with the sites of tumor. Early clinical studies reported by Reif et al, *J. Surg.Oncol.*, 6, 133 (1974) and Mach et al, *Europ. J. Cancer, Suppl.* 1, 113 (1978) failed to show tumor localization in humans with radioactive anti-CEA antibodies.

Goldenberg et al, in an article in the *New England Journal of Medicine*, 298, 1384 (1978), reported success in clinical trials of tumor detection and localization by scintillation scanning of patients receiving radiolabeled antibodies to CEA. In that reference, it was noted that there was a problem in both animal and human studies in distinguishing specific radioantibody activity from blood-pool background activity, and that special scanner subtraction techniques with other radionuclides were considered essential for unequivocal tumor localization using this method. The antibody preparation used in the reference was 70% immunoreactive with CEA. The reference further notes that the absence of CEA in normal hamster tissues precludes extrapolation to man, in whom the antigen usually circulates in increased levels in patients with cancer, and is present in lesser quantities in certain normal tissues. The subtraction technique used to permit localization using this scintigraphic method involved injection of Tc-99m-pertechnetate and Tc-99m-labeled human serum albumin prior to each imaging scan. The data obtained were stored in a minicomputer capable of generating digital images of the labeled antibody alone, the Tc-99m labeled species together, and sums and differences of these various values.

Even this most recent and successful tumor localization and detection process has certain disadvantages which limit its resolution, it efficiency and its practicability. The use of a different radionuclide attached to a carrier having kinetics of transport and distribution different from an antibody in the subtraction technique used to distinguish tumor localized antibody from background activity is not an ideal procedure. Furthermore, the need to inject these materials prior to each photoscan is an inconvenience, it not an ideal procedure, and exposes the patient to increased levels of radioactivity. U.S. Pat. No. 3,927,193 teaches that the anti-CEA antibody should not be labeled to a degree which might interfere with the activity of the antibody, a limitation which was not questioned in the later references discussed above. However, this limits the resolution of the method and requires larger quantities of antibody for image detection.

Tumor radiotherapy using labeled antibodies has been suggested by many, and an indication of its success in a single multimodal therapeutic clinical use is reported by Order, *Radiol.*, 118, 219 (1976). The use of boron-labeled antibodies in therapy is reported by Hawthorne et al., *J. Med. Chem.*, 15, 449 (1972), but the combined incorporation of boron and a radioisotope for localization is not suggested.

A need therefore continues to exist for a method of tumor detection and localization which can achieve high resolution and which avoids the aforementioned disadvantages.

OBJECTS OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method of tumor localization and detection which achieves high resolution without the necessity of repeated injection of other radioactive material for computer subtraction of background activity.

Another objective of the present invention is to provide an antibody for tumor detection and localization having a high specific activity and a high specificity for CEA, thereby improving the resolution of scintigraphic tumor localization and detection methods.

A further object of the invention is to provide a method of tumor radiotherapy wherein a radiotherapeutically effective radioisotope is concentrated at the site of tumor growth by virtue of its attachment to an antibody which is highly specific to carcinoembronic antigen.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the art.

SUMMARY OF THE INVENTION

The foregoing objects are achieved by providing, in a method for determining the location of a tumor which either produces or is associated with carcinoembryonic antigen (CEA), which comprises injecting a subject parenterally with an antibody specific to CEA and radiolabeled with a pharmacologically inert radioisotope capable of detection using a photoscanning device and subsequently scanning the subject with said device to determine the location of the resultant uptake of said antibody by said tumor; the improvement which comprises concurrently injecting said subject with normal immunoglobulin from the same or different species as that used to prepare said specific antibody, said normal immunoglobulin being radiolabeled with a different radioisotope of the same element used to label the specific antibody and emitting at an energy capable of independent detection using said photoscanning device, the level of activity of the labeled normal immunoglobulin being used to determine the distribution of background activity due to non-targeted specific antibody, said distribution being subtracted from the total activity of specific antibody whereby the activity of substantially only the targeted tumor-associated antibody is determined.

The invention further provides an improvement in the foregoing general method which comprises using as said anti-CEA antibody a substantially monospecific antibody having a CEA-specific immunoreactivity prior to labeling of at least 70% and a cross-reactivity of other antigens of less than 15%, said antibody being radiolabeled to an extent sufficient to reduce its CEA-specific immunoreactivity by from 5 to 33%. An antibody and an injectable composition suitable for use in the foregoing method are provided, as are methods of tumor radiotherapy using radiolabeled anti-CEA antibody.

ministration, and the timing of immunization, as well as the interval between the last booster injection and the removal of the spleen cells.

The spleens are removed and placed in 60 mm Petri dishes containing either serum-free medium or Dulbecco's Modified Eagle's Medium (DMEM) with 20% fetal calf serum, at room temperature, and minced with scissors to disperse the cells. The cells are further liberated by agitation for 1–2 min on a Vortex mixer. The spleen cells are removed to a conical centrifuge tube and pelleted at 1,000 rpm in an IEC-MS2 centrifuge, the supernatant is removed, the pellet tapped loose, and then resuspended in 5 ml of cold 0.17 NH_4Cl for 10 min to lyse red blood cells. Chilled DMEM with 20% fetal calf serum is added and the cells pelleted, and then again suspended in 10 ml DMEM supplemented with 20% fetal calf serum.

The myeloma cell lines used for fusion are maintained in stationary suspension cultures in DMEM with high glucose (4.5 g/L) and 20% fetal calf serum, in 5–10% CO_2 at a cell concentration between 100,000 and 1,000,000 per ml. The myeloma (plasmacytoma) cell lines can be P3/X63-Ag8, which is a Balb/C plasmacytoma derived from MOPC-21 (Svasti and Milstein, Biochem. J. 128: 427–444, 1972), or a derivative thereof known as FO (Fazekas de St. Groth and Scheidegger, Basle Institute of Immunology, Basle, Switzerland), or 45.6TG1.7, which is a Balb/C line derived from MPC-11 (Margulies et al., Cell 8: 405–415, 1976). All of these lines lack the enzyme hypoxanthine phosphoribosyl transferase (HPRT; E.C. 2.4.2.8) and are thus killed in a selective medium containing hypoanthine, aminopterin, and thymidine (HAT), as described by Littlefield (Science 145: 709–710, 1964).

The spleen cells obtained from the immunized animal are then fused with the plasmacytoma cells by using polyethylene glycol according to an adaptation of the method of Gelfer et al. (Somatic Cell Genetic. 3: 231–236, 1977). For example, a 30% polyethylene glycol solution is made by heating sterile polyethylene glycol 4000 (Merck, molecular weight of about 4,000) (0.5 g Polyethylene glycol+0.05 ml dimethyl sulfoxide (DMSO)+0.5 ml distilled water) and DMEM without serum to 41° C. and mixing 3 ml of polyethylene glycol with 7 ml DMEM without serum, pH 7.4–7.6, and kept at 37° C. until use. Fusions are made at room temperature. The myeloma cells (10^6–10^7) are washed twice in serum-free medium and then mixed with $1–3\times10^7$—$1–3\times10^8$ spleen cells in 50 ml conical bottom centrifuge tubes (Falcon 2070). The cells are centrifuged at 250×g for 5 min, and the supernatant fluid is carefully aspirated. An amount of 0.2 ml of the polyethylene glycol preparation is added, and the tube is gently agitated by hand to resuspend the cells. Next, the cells are centrifuged for 3 min at 250×g and again at 400×g for another 3 min, and then kept undisturbed for an additional 3 min. The cells are exposed to polyethylene glycol for about 8 minutes. Thereafter, about 5 ml of serum-free medium is added to the tube, the cells are resuspended gently, and then repelleted by centrifugation at 250×g for 5 min. The supernatant is removed and the cells are suspended in 20 ml of serum-containing medium and incubated at 37° C. in a humidified incubator for 48 hr. before being placed in microplates to which HAT medium is added. Alternatively, the cells are immediately suspended in 30 ml of a medium consisting of DMEM, 10% NCTC 109 medium (Microbiological Associates), 20% fetal calf serum (GIBCO), 0.2 units bovine insulin/ml (Sigma), 0.45 mM pyruvate, 1 mM oxaloacetate, and antibiotics of choice. Thymidine (1.6×10^{-5} M) and hypoxanthine (1×10^{-4} M) are added. The cells in this medium are distributed into 6 microplates (Linbro FB 96 TC) with 1 drop (about 50 μl) per well. The next day 1 drop of the above-specified medium containing thymidine and hypoxanthine, now with aminopterin (8×10^{-7} M), is added to each well. Two drops of the medium of above is added 6–7 days later and clones appear microscopically between 10 and 20 days. The hypoxanthine-aminopterin-thymidine (HAT) medium can also be added immediately after the fusion, or at a later time. An improvement in the number of hybrids obtained is made when a feeder layer is added to each microwell. Here, human fetal fibroblasts are irradiated with 4500 r, and 1,000–2,000 such cells are added to each well, either the day before the fusion or directly to the fused cells and so dispensed with them into the microwells. After clones have appeared macroscopically, the medium is changed by removing most of the medium and adding fresh medium. After a second change of medium, the medium is left there for at least 4 days and then collected for assays of antibody activity and specificity by conventional assays.

Large amounts of antibody are obtained from spent culture medium harvested from 150 mm plates or roller bottles. The medium is subsequently concentrated by means of a hollow-fiber concentrator (Amicon). Also, antibody is obtained from the ascites fluid of athymic (nude) mice (nu/nu) that were injected 2–3 weeks previously with about 1 billion cloned hybridoma cells. The ascites fluid is diluted with saline by flushing the peritoneal cavity of each mouse with saline, the diluted fluids from each mouse are pooled.

The monoclonal anti-CEA IgG is radiolabeled with I-131 as in Example 1(f).

EXAMPLE 3

Preparation of ^{123}I-IgG (goat)

Normal goat immunoglobulin G (IgG) (Miles) is affinity purified against cyanogen bromide-linked CEA and labeled with I-123 as in Example 1(f), except that I-123 is substituted for I-131, with proportional changes in the reagents to account for differences in specific activity.

EXAMPLE 4

Preparation of ^{131}I-anti-CEA-^{10}B IgG

(a) Anti-CEA IgG prepared according to Examples 1 or 2 is reacted with a 20-fold molar excess of the diazonium salt of 1-(4-aminophenyl)-1,2-dicarba-closo-dodecaborane (12) having a natural abundance of Boron-10 isotope (20%), using the procedure of Hawthorne et al., *J. Med. Chem.*, 15, 449 (1972). The resultant antibody has an average of from 2 to 10 diazo-linked carborane residues or from 4 to 20 Boron-10 atoms per antibody molecule.

(b) The anti-CEA-^{10}B of part (a) is radiolabeled with I-131 as in Example 1(f), to introduce an average of from 2.5 to 10 atoms of iodine per antibody molecule.

EXAMPLE 5

Preparation of injectable compositions

Sterile, pyrogen-free solutions are prepared as shown.

(a) A sterile solution containing, per ml:

(1) 10 mg Human Serum Albumin (HSA) (1%, USP, Parke-Davis)
(2) 0.01 M phosphate buffer, pH 7.5 (Bioware)
(3) 0.9% NaCl
(4) 80 μg ^{131}I-anti-CEA IgG (goat) prepared according to Example 1 (average of about 5 atoms of iodine/molecule, specific activity of about 40 μCi/μg).

The labeled antibody of Example 1 is stored in a solution of (1), (2) and (3) at a concentration of 160 μg/ml and diluted with an equal volume of 1% HSA in phosphate buffered saline (PBS) to prepare this solution.

(b) A sterile solution according to the procedure of part (a) except that it further contains 80 μg/ml of ^{123}I-IgG as prepared in Example 3. The ^{123}I-IgG is stored in phosphate buffered saline containing 1% HSA at a concentration of 160 μg/ml. An equal volume of this solution is used in place of 1% HSA in PBS in the procedure of part (a).

(c) A sterile solution according to the procedure of part (b) except that the antibody is the ^{131}I-anti-CEA IgG (monoclonal) prepared according to Example 2, stored in 1% HSA in PBS at a concentration of 160 μg/ml and having comparable activity.

(d) A sterile solution according to the procedure of part (b) except that the antibody is the ^{131}I-anti-CEA-^{10}B IgG prepared according to Example 4, having an average of 5 diazo-linked carborane residues and 3 atoms of iodine per antibody molecule, and a specific activity of about 24 μCi/μg. The final solution contains 133 μg/ml of the antibody.

EXAMPLE 6

Tumor Localization

Radioiodinated anti-CEA IgG is administered to patients with suspected tumors. The patient is pre-tested for anaphylactic hypersensitivity to goat IgG or myeloma IgG. To block thyroid uptake of I-131 or I-123, Lugol's solution (Purepack) is administered by mouth, 5 drops twice daily for seven days beginning one day before injection of the radioactively labeled antibody.

Localization is effected according to the procedure of Goldenberg et al., *N. Eng. J. Med.* 298, 1384 (1978), by infusion of a 0.06 ml solution of ^{131}I-anti-CEA IgG containing ^{123}I-IgG prepared according to Example 5(b) or 5(c) in 20 ml of sterile physiological saline over a period of from 10 minutes to 45 minutes. No Tc-99m compounds are used, the subtraction technique being adapted in a conventional fashion to discriminate between ^{131}I and ^{123}I. Scans are taken immediately and at 2, 8, 12, 24, 48, and 72 hours after injection of the antibody is completed.

Significant localization is seen after 2 hours, with improved resolution with time, tending to plateau between 8 and 24 hours after injection. No additional background ^{123}I-IgG is added. The CEA-selectivity of this method is comparable to the earlier Goldenberg et al. method, but the resolution, rapidity and convenience is enhanced significantly.

EXAMPLE 7

Tumor Therapy

(a) A patient having an ovarian cancer, optionally detected and localized by the procedure of Example 6, is injected by intravenous infusion with 150 mCi of the solution of Example 5(a) in 50 ml of sterile physiological saline. Reduction in tumor size is observed within 20 days. The dose is repeated at intervals adjusted on an individual basis.

(b) A patient having a cervical cancer optionally detected and localized by the procedure of Example 6 is injected with an amount of the solution of Example 5(d) (in 50 ml of sterile physiological saline) sufficient to provide 200 μCi of ^{131}I activity based on a 70 kg patient weight.

The tumor is precisely localized 12 hours after injection using the procedure of Example 6. A well collimated beam of thermal neutrons is focused on the defined tumor locations. Irradiation with an external neutron beam dose of 400–800 rads, delivered in a period of from 8–20 min, is effected for each tumor locus, and is optionally repeated with administration of the tumor-localizing antibody, with or without the radiolabel, at intervals adjusted on an individual basis, but usually not exceeding a total dose of 3200 rads unless simultaneous external neutron beam therapy is indicated.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed is:

1. In a method for determining the location of a tumor which either produces or is associated with carcinoembryonic antigen (CEA), which comprises injecting a human subject parenterally with an antibody specific to CEA and radiolabeled with a pharmacologically inert radioisotope capable of detection using a photoscanning device and subsequently scanning the subject with said device to determine the location of the resultant uptake of said labeled antibody by said tumor;

the improvement which comprises concurrently injecting said human subject with normal immunoglobulin from the same or different species as that used to prepare said specific antibody, said normal immunoglobulin being radiolabeled with a different radioisotope of the same element used to label the specific antibody and emitting at an energy capable of independent detection using said photoscanning device, the level of activity of the labeled normal immunoglobulin being used to determine the distribution of background activity due to non-targeted specific antibody, said distribution being subtracted from the total activity of specific antibody whereby the activity of substantially only the targeted tumor-associated antibody is determined.

2. The method of claim 1, wherein the specific anti-CEA antibody is labeled with one of, and the normal immunoglobulin is labled with the other of Iodine-131 and Iodine-123; Indium-111 and Indium-113m; Gallium-67 and Gallium-68; Ruthenium-97 and Ruthenium-103; or Mercury-197 and Mercury-203.

3. The method of claim 1, wherein the specific anti-CEA antibody is labeled with one of Iodine-131 or Iodine-123 and the normal immunoglobulin is labeled with the other of Iodine-131 or Iodine-123.

4. The method of claim 1, wherein the amount of radiolabel introduced into the specific anti-CEA anti-

other antigens of less than 15%, said antibody being radiolabeled with a pharmacologically inert radioisotope capable of detection using a photoscanning device, the extent of radiolabeling being sufficient to reduce said CEA-specific immunoreactivity by from 5 to 33%; and

(b) a pharmaceutically acceptable injection vehicle.

28. The composition of claim 27, wherein said CEA-specific immunoreactivity is at least 80% and said cross-reactivity is less than 10%.

29. The composition of claim 28, wherein said radioisotope is Iodine-131 or Iodine-123, an average of at least 5 atoms of iodine per antibody being introduced.

30. The composition of claim 27, wherein said antibody is a monoclonal anti-CEA antibody.

31. The composition of claim 30, wherein said radioisotope is Iodine-131 or Iodine-123, an average of at least 5 atoms of iodine per antibody being introduced.

32. A method of tumor radiotherapy, which comprises parenterally injecting into a human subject having a tumor which produces or is associated with carcinoembryonic antigen (CEA) a tumor-reducing amount of an antibody which is specific to CEA and radiolabeled with a pharmacologically inert, radiotherapeutically effective radioisotope; wherein said antibody is substantially monospecific to CEA, having a CEA-specific immunoreactivity prior to labeling of at least 70% and a cross-reactivity to other antigens of less than 15%.

33. The method of claim 32, wherein said antibody is a monoclonal anti-CEA antibody.

34. The method of claim 32, wherein said radioisotope is I-131 and said amount is from 25 to 250 mCi per administration.

35. The composition of claim 27, which further comprises normal immunoglobulin from the same or different species as that used to prepare said specific antibody, said normal immunoglobulin being radiolabeled with a different isotope of the same element used to label the specific antibody and emitting at an energy capable of independent detection using said photoscanning device.

36. A method of tumor radiotherapy, which comprises the steps of parenterally injecting into a human subject having a tumor which produces or is associated with CEA a radiotherapeutically effective amount of an antibody which is specific to CEA and radiolabeled with a pharmacologically inert radioisotope capable of detection with a photoscanning device, said labeled antibody further containing in chemical combination an addend containing at least five atoms of boron with at least a natural abundance of Boron-10 isotope; locating said tumor by scanning the subject with said photoscanning device to determine the location of the resultant uptake of said labeled antibody by said tumor; and directing a beam of thermal neutrons at said tumor location.

37. The injectable composition of claim 24 or 27, wherein said radiolabeled anti-CEA antibody further contains in chemical combination an addend containing at least five atoms of boron with at least a natural abundance of Boron-10 isotope.

38. An antibody which is specific to carcinoembryonic antigen, said antibody being radiolabeled with a pharmacologically inert radioisotope capable of detection with a photoscanning device, said labeled antibody further containing in chemical combination an addend containing at least five atoms of boron with at least a natural abundance of Boron-10 isotope.

39. The antibody of claim 17, wherein said radiolabeled antibody further contains in chemical combination an addend containing at least five atoms of boron with at least a natural abundance of Boron-10 isotope.

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